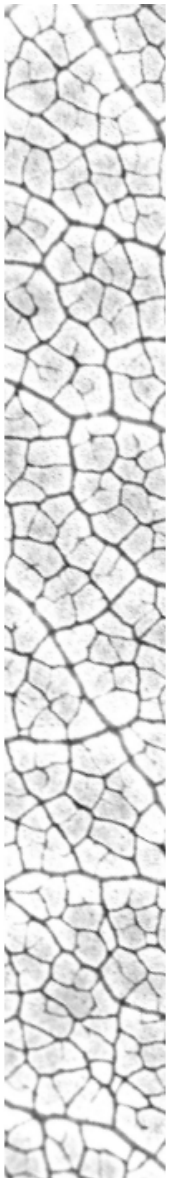

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SOCS (Suppressor of Cytokine Signaling) protein as material to enhance the effect of growth hormone in broilers

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Abstract
The purpose of this study was to identify specific proteins that can slow down the SOCS protein activity in STAT signaling protein termination so that it will induce the increase in GH metabolic effect in enhancing the growth of broilers and their meat quality. It is, therefore, necessary to identify the molecular weight and the composition of the SOCS (Suppressor of Cytokine Signaling) protein acid that act in the signaling of STAT (Signal Transducers and Activators of Transcription) activated by GH (Growth Hormone) in broilers. By identifying the molecular weight and the composition of the amino acids of SOCS proteins, there is a great chance to make a specific protein that can slow down the SOCS protein activity in STAT signaling protein termination. Therefore, there is an increase in GH metabolic effect in enhancing the broilers' growth and their meat quality. The results indicated that SOCS-1 protein was present in broilers' liver tissue. An examination with Western Blot noted that the molecular weight of the SOCS-1 protein was 98 kDa. This finding signifies that SOCS-1 protein is a protein that serves as negative feedback on broiler growth through growth hormone.

Keywords: protein SOCS, growth hormone, growth, broiler

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INTRODUCTION

Growth hormone (GH) has significance in the regulation of growth and metabolism. GH receptor is a member of the cytokine receptor family known to be associated with and to activate tyrosine kinase (Endo et al. 1997). The binding of GH to its receptor can activate Janus Kinase 2 (JAK 2) and further phosphorylate tyrosine in the JAK-2 GH-receptor complex. This tyrosine then forms the bonding site for many signaling proteins, such as signals of transducers and activators of transcription (STAT).

Among various signaling molecules previously mentioned, the STAT protein plays a significant role in GH regulation and transcription. The growth hormone is known to activate STAT-1, STAT-3, STAT-5a, and STAT-5b. The cytoplasmic STAT proteins form a complex with other STAT proteins through phosphorylated tyrosine interactions in the SH-2 domain, transmit translocation to the nucleus, bind to DNA, and then activate the transcription of the target gene to spur growth (Ihle 1996).

GH-enabled STAT signaling protein terminations involve two types of signaling events: (1) tyrosine dephosphorylation in GHR, JAK2, and STATs by phosphatase, (2) protein suppressors of cytokine signaling (SOCS) expression. GH-enabled STAT signaling protein terminations require tyrosine phosphatase activation in the GHR/JAK2 complex. This phosphatase further dephosphorylates GHR, JAK2, and STAT to perform down-regulatory signaling. Because STAT requires phosphorylated GHR for docking and subsequent activation by JAK2, GHR dephosphorylation is suspected to terminate STAT activation. GHR dephosphorylation also marks the occurrence of degradation (Gebert et al. 1999). Tachosine dephosphorylation in the JAK2 kinase domain is believed to inactivate JAK2, whereas tachosine dephosphorylation in STAT will inhibit DNA binding so that it will terminate signaling.

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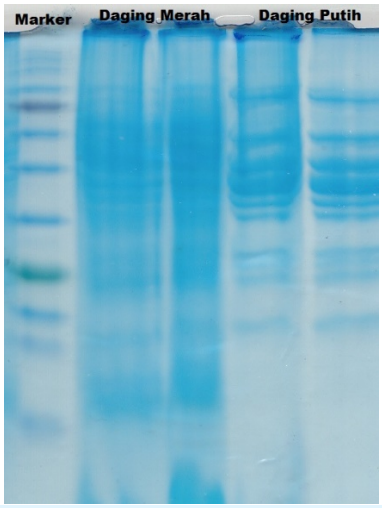

Fig. 1. SDS-Page from broilers' liver

The phosphatase that acts as a negative regulator of STAT-activated STAT signaling proteins is SHP-1 and SHP-2, which contains the SH2 domain. SHP-1 is known as a negative signaling regulator of JAK/STAT-mediated by cytokine receptors in hematopoietic cells. SHP-1 will be associated with JAK2 to cause JAK2 deficiency in the liver. SHP-1 and SHP-2 are considered potential phosphatase for STAT 5 (Yu et al., 2000). SHP-2 associates with GHR in response to GH, a tyrosine residue mutation in GHR that serves as a binding site of SHP-2 will prolong the phosphorylation of tyrosyl GHR, JAK2, and STAT-5b so that the GH metabolic effect lasts longer (Stofega et al. 2000).

SOCS protein acts as negative feedback in cells. Its expression is induced by cytokines or hormones and acts to inhibit the signaling of activated receptor complexes (Naka et al. 1997). The growth hormone induces the expression of SOCS-1, SOCS-2, SOCS-3 and CIS in mice livers in different degrees (Ram and Waxinan 1999). The signaling pathway involved in the induction of SOCS expression in response to GH remains unclear. However, it is reckoned to require signaling protein STAT (Naka et al. 1997).

SOCS-1 can interact directly with JAK (Endo et al. 1997). The SOCS-1 inhibition of JAK2 activity requires the interaction between SH2, SOCS-1, and JAK2 domains (Yasukawa et al. 1999). It is estimated that the N-terminal region of the SH2 domain acts as a JAK2 pseudosubstrate inhibitor. SOCS-1 inhibits JAK2 tyrosyl phosphorylation and then subsequently and constitutively terminates STAT5B tyrosyl phosphorylation, DNA binding, and STAT5B-mediated gene expression (Ram and Waxman 1999).

It is therefore expected that SOCS protein structure can be identified for specific proteins that can inhibit STAT signaling termination so that GH metabolic effect can be extended. This notion is the underlying importance of this research to be implemented immediately.

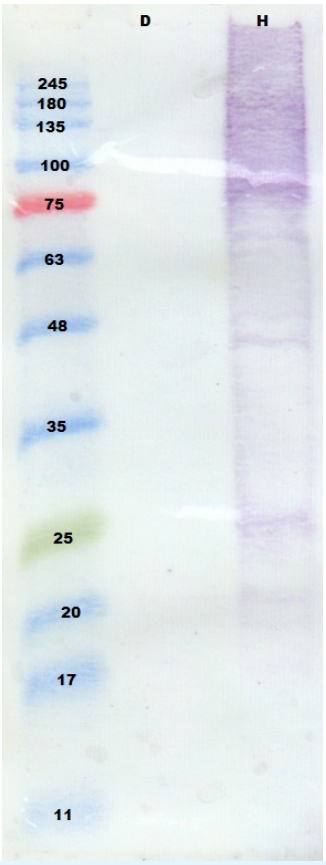

Fig. 2. Result of Western Blot for SOCS protein from broilers' liver

RESULT AND DISCUSSION

SDS Page of the Broilers' Meat and Liver

The result of SDS-Page from broilers' liver suggested that there was SOCS protein as shown in **Fig. 1**.

This result indicated that a clear protein band was formed between 75 kDa and 100 kDa markers. The protein bands between 75 kDa and 100 kDa markers were suspected of SOCS protein. However, further examination using a Western Blot test is required to confirm.

Western Blot for SOCS Protein from Broilers' Liver

The results of SDS-PAGE for liver tissue proteins revealed that the most obvious protein bands between 75 kDa and 100 kDa markers were suspected as SOCS protein. However, the result of the test had not determined that it was a SOCS protein. To prove that the formation of the protein bands was SOCS protein, it is necessary to make a further examination by using the Western blot test.

To determine that the result of protein analysis with SDS-PAGE and SOCS protein, the Western blot test was conducted by using rabbit polyclonal antibody SOCS Ab-1 (Labvision). **Fig. 2** indicates the formation of

one of the clearest protein bands between 75 kDa with 100 kDa markers. Both proteins were formed in hepatic tissue, and the molecular weight was found to be 98 kDa. This suggests that the SDS-PAGE protein tested with Western blot was a SOCS protein from growing phase broiler with a molecular weight of 98 kDa. The formation of a protein band with 98 kDa molecular weight was definite because of the binding between SOCS protein resulted from SDS-PAGE and rabbit polyclonal antibody SOCS.

GH-enabled STAT signaling protein terminations involve two types of signaling events: (1) tyrosine dephosphorylation in GHR, JAK2, and STATs by phosphatase, and (2) protein suppressors of cytokine signaling (SOCS) expression. The GH-enabled STAT signaling protein terminations require tyrosine phosphatase activation in the GHR/JAK2 complex. This phosphatase further causes dephosphorylate GHR, JAK2, and STAT to perform down-regulate signaling. As STAT requires phosphorylated GHR for docking and subsequent activation by JAK2, the GHR dephosphorylation is suspected to terminate STAT activation. GHR dephosphorylation also marks the occurrence of degradation (Gebert et al. 1999). Tachosine dephosphorylation in the JAK2 kinase domain is believed to inactivate JAK2, whereas the tachosine dephosphorylation in STAT will inhibit DNA binding so that it will terminate signaling.

The phosphatases, which act as a negative regulator of STAT-activated STAT signaling proteins, are SHP-1 and SHP-2 which contain the SH2 domain. SHP-1 is known as a negative signaling regulator of JAK/STAT, which is mediated by cytokine receptors in hematopoietic cells. SHP-1 is associated with JAK2 to defoil JAK2 in the liver. SHP-1 and SHP-2 are considered as potential phosphatases for STAT 5 (Yu et al. 2000). SHP-2 is associated with GHR in response to GH, a tyrosine residue mutation in GHR that serves as a binding site of SHP-2 which will prolong the phosphorylation of tyrosyl GHR, JAK2 and STAT5B so that the GH metabolic effect lasts longer (Stofega et al. 2000).

Other phosphatases suspected to be involved in GHR dephosphorylation, JAK2, and STAT, are serine/threonine inhibitors H-7 and cycloheximide, which will slow down the inactivity of JAK2 (Gebert et al. 1999).

SOCS protein acts as negative feedback in cells. Its expression is induced by cytokines or hormones and acts to inhibit the signaling of activated receptor complexes (Naka et al. 1997). GH induces expression of SOCS-1, -2, -3 and CIS in mice liver in different degrees (Ram and Waxinan, 1999). The signaling pathway involved in the induction of SOCS expression in response to GH remains unclear but is thought to require STAT signaling protein (Naka et al. 1997).

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The action of SOCS-2 on GH-induced STAT signaling proteins is not identified. As for SOCS-3, it is rapidly induced in the liver by GH (Tollet-Egnell et al. 1999). This notion suggests that SOCS-3 plays an important role in STAT signaling protein termination. In contrast to SOCS-1, SOCS-3 inhibits JAK2 through a mechanism that requires GHR (Hansen et al. 1999). Nevertheless, the mechanism of this process is not yet clear. Research using the telosyl tyrosylated GHR cytoplasm domains in bacteria by a kinase other than JAK2 indicates that SOCS-3 and other SOCs may bind directly to GHR. This suggests the importance of using JHF phosphorylated GHR2 in determining the JAK2 inhibition mechanism by SOCS.

Like SOCS-3, CIS is particularly induced in the liver by GH and also requires GHR to inhibit JAK2 (Ram and Waxman 1999). By the CIS physiological significance of GH signaling, transgenic mice expressing CIS have reduced weight and a major urinary protein (MUP) level in their urine (Matsumoto et al. 1999). This fact is the characteristics of STAT5 deficiency mice.

MATERIALS AND METHODS

Day old chickens were placed in a cage for 14 days with *ad libitum* feed. At the age of 15 days, the chickens were then placed in a battery enclosure with a capacity of one chicken per cage, fed twice a day at 6.00 AM and 6.00 PM in the amount of 10% less than standard. At the age of 21 days, they were sacrificed for their hepatic tissue intended for the following examination steps. First, the isolation of the SOCS signaling protein was extracted from broiler liver tissue. Next, the isolated SOCS signaling protein was identified using SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoreses) method. Last, the molecular weight of SOCS signaling protein was analyzed by the Western blotting method using electrophoresis elucidated protein from a polyacrylamide gel.

SDS-PAGE FOR SOCS PROTEIN

Gel Preparation

The gel plate was prepared by arranging two glass plates in a distance of approximately 1 mm. The gel was made of two layers, one for collecting the samples (stacking gel) and the other one as a medium for separating the protein (separating gel). The separating gel 12% (1 plate) was prepared by adding a 1,700 µL distilled water, 1,300 µL LGB (lower gel buffer), and

2,000 µL T-acyl 30%, then confirmed for 10 minutes. Next, 70 µL APS (ammonium persulfate) 10% and 7 µL TEMED were added sequentially. Then, the beaker glass was shaken slowly to flatten all solutions. The solution was then poured into the plate using a pipette up to 3/4 height of the plate. The water was poured gently over the gel solution so that the gel surface was not wavy. While waiting for the separating gel to solidify, the 3% (1 plate) stacking gel was prepared by using 975 µL distilled water, 415 µL UGB (upper gel buffer), 267 µL T-acyl 30%, 20 µL APS 10%, and 2 µL TEMED. The beaker glass was shaken slowly and immediately set after the solution was poured into the plate. The sample forming comb was slowly inserted. After the gel solidified the comb was lifted, and then the sample filling was done on the gel wells.

Injection of Protein Samples

A total of 20 µL protein and protein solutions were added, each with 20 µL sample buffer (RSB or reducing sample buffer) and then heated into a water heater at 100° C for 3 minutes. After cooling down, the sample was ready to put into the gel wells in a volume of 24 µL for each well. The standard protein used was the standard broad range of protein (BioRad).

Western Blotting for SOCS

The SDS-PAGE gel sheets containing the protein bands were transferred to nitrocellulose paper using a Biorad artificial dry blotter instrument and then transferred to nitrocellulose by 300 mA for 30 minutes. Then, they were stained with 2% poncho containing TCA until the concentration reached 3%. Unspecific protein was blocked on 3% BSA in a TBE solution with a pH level of 7.4 and 0.05% Tween 20 and then incubated overnight. Then, it was washed on a TBE solution with a pH level of 7.4 plus 0.05% Tween 20 twice, each for 5 minutes. It was then incubated in Rabbit Anti-Phospho-STAT 1 (Tyr694) primary antibody in 5 µg/mL concentration in TBE solution with a pH level of 7.4 containing 1% concentration of BSA solution. Then it was shaken for 2 hours. Subsequently, it was washed with a TBE solution with a pH level of 7.4 containing 0.05% Tween 20. It was then incubated in biotin-labeled secondary antibody in 1/1,000 concentration in TBE solution with a pH level of 7.4 with 1% BSA. It was washed again with TBE solution with a pH level of 7.4 containing 0.05% Tween 20, then incubated with SA-HRP for 40 minutes and washed with TBE solution with a pH level of 7.4 and 0.05% Tween 20 twice, each for 5 minutes. Visualization was done on tetramethylbenzidine (TMB) for 30 minutes. It was rinsed with H2O twice, each for 5 minutes.

CONCLUSION

The suppressors of cytokine signaling (SOCS) protein can be found in broilers' liver. The molecular weight of suppressors of cytokine signaling (SOCS) protein is 98 kDa.

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